Steroidal Sapogenins. IV.[1] Hydrolysis of Steroidal Saponins[2]

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Steroidal sapogenins are recognized as excellent sources for sex hormones and may be important precursors for cortisone synthesis.[3] The sapogenins are not found free, but occur in a combined glucosidal form which can be cleaved by the use of strong hydrochloric acid. Consequently, the yield of sapogenin depends on the completeness of the acid hydrolysis of the precursor saponins. The question of yield has not been clearly elucidated by previous workers. Jurs and Noller[4] hydrolyzed a purified saponin precursor of tigogenin (isoallospirostan-3β-ol) using 1.4 *N* hydrochloric acid for 72 hours. Marker, *et al.*,[5] hydrolyzed crude alcoholic plant extracts with 2 *N* hydrochloric acid for 2–3 hours. In neither case was it shown that the hydrolysis conditions were optimal.

We have studied the acid hydrolysis of a number of different saponins. The results are summarized in Table I. Several important facts can be deduced from the data. All the saponins tested were rapidly hydrolyzed with 4 *N* hydrochloric acid, yielding in most cases 90% of the total sapogenin found, in 1–2 hours. Hydrolysis occurred very rapidly when 6 *N* acid was used, cleavage taking place within 10 minutes with the two saponins tested.

Hydrolysis of the different saponins with 2 *N* acid resulted in variable hydrolysis rates. In all cases, the time to reach 90% hydrolysis was much longer than the comparable 4 *N* period, and as shown in Table I, the times varied from 2 hours to more than 8 hours.

In all the experiments, use of 1 *N* hydrochloric acid was ineffective, as was also hydrolysis with 1 and 2 *N* sulfuric acid (not shown in table). Destruction of sapogenins with excess heating time apparently occurred only when 6 *N* hydrochloric acid was used.

The limited data indicate that the structure of the steroidal aglucone portion of the molecule does not influence the rate of hydrolysis (*i.e.*, isomerism at C_5 or C_{22} or number of hydroxyl groups). This is best shown by the data obtained with 2 *N* acid hydrolysis. Sarsasaponin, yielding spirostan-3β-ol and gitonin, the precursor of isoallospirostan-2α,3β-diol, are the most rapidly hydrolyzed saponins. Dioscin, yielding Δ^5-isospirosten-3β-ol, and digitonin forming isoallospirostan-2,3β,15(?)-triol, were more resistant to hydrolysis. Chloronin, forming isoallospirostan-3β,6-diol, was the most difficultly hydrolyzable saponin tested.

The results discussed above suggest that the routine use of 2 *N* hydrochloric acid for the hydrolysis of *unknown saponins* in crude plant extracts can result in *low sapogenin yields*. Crude plant extracts such as those used by Marker, *et al.*,[5] contain proteins and sugars. It is not surprising therefore, that when we attempted to hydrolyze such extracts with 4 *N* acid, large quantities of tar were produced, from which little sapogenin could be isolated. Subsequently, a procedure was developed at this Laboratory[6] in which saponins could be routinely separated from proteins and carbohydrates by extraction from the aqueous phase with butanol. After this treatment, the saponin preparations could be hydrolyzed by refluxing with 4 *N* acid for 3–4 hours with little tar formation. The sapogenins thus formed are readily isolated. A number of experiments comparing the direct 2 *N* hydrolysis[5] with the butanol purified 4 *N* hydrolysis[6] have invariably shown that the latter procedure gives 25–100% higher yields of sapogenin.

Experimental

Purified saponin preparations were prepared as described previously[1,6] with the exception of digitonin, which was ob-

(1) Paper III, E. S. Rothman, M. E. Wall and C. R. Eddy, This Journal, **74**, 4013 (1952).

(2) Not copyrighted.

(3) For pertinent references of papers by Marker, *et al.*, This Journal, (1940–1947), and Djerassi, Rosenkranz, *et al.*, This Journal, (1950–1952).

(4) P. C. Jurs and C. R. Noller, *ibid.*, **58**, 1251 (1936).

(5) R. E. Marker, *et al.*, *ibid.*, **69**, 2167 (1947).

(6) Paper I, M. E. Wall, M. M. Krider, E. S. Rothman and C. R. Eddy, *J. Biol. Chem.*, in press.

TABLE I

ACID HYDROLYSIS OF STEROIDAL SAPONINS

Saponin and derived sapogenin	Hydrochloric acid normality	Percentage of total sapogenin (based on 4 *N*) Time (hours) 1/6	1/2	1	2	3	4	5	6	8	72	Time for 90% hydrolysis, hours
Sarsasaponin spirostan-3β-ol	1				27	47	53	60	67			>6
	2			47	73	87	93		93			3–4
	4		80	87	93	93	93		100			1–2
Dioscin Δ^5-isospirosten-3β-ol	1			13	17		17	26				>5
	2			9	35	70	74	78				>5
	4		74		83	91	100	100				3
Chloronin isoallospirostan-3β,6α-diol	2						41			57	99	>8
	4				93		98			100		1–2
	6	93					61			63		<1
Digitonin isoallospirostan-2,3β,15(?)-triol	1				12	18	30	35				>5
	2				47	65	80	84				>5
	4			89	91	100	100	100				$^1/_2$–1
Gitonin isoallospirostan-2α,3β-diol	2			82	89		94					2
	4			89	98		100					1
	6	100	77									<1

tained from a commercial source. The saponin yielding isoallospirostan-3β,6α-diol was obtained from bulbs of *Chlorogalum pomeridianum;* the saponin yielding Δ^5-isospirosten-3β-ol was obtained from rhizomes of *Dioscorea composita;* the saponin yielding isoallospirostan-2α,3β-diol[7] was obtained from leaves of *Yucca gloriosa;* and the saponin yielding spirostan-3β-ol was obtained from the leaves of *Yucca baccata.*[8]

Stock solutions of saponins were prepared in 1:1 ethanol–water (by volume) so that 3.34-ml. aliquots contained 100 mg. of saponin. To the aliquots were added sufficient concentrated hydrochloric acid, water and alcohol to bring the final volume of 5.00 ml. to the desired normality. Two ml. of benzene, previously equilibrated with an equal volume of 1:1 ethanol–water was added, and the hydrolyses were conducted at reflux temperature in centrifuge tubes immersed in a water-bath at 75–78° as described previously[9] and the sapogenins, as acetates, were assayed by the infrared method,[9,10] or in some cases gravimetrically.

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(7) A small quantity of isoallospirostan-3β-ol was also present.

(8) We wish to thank C. O. Erlanson, D. S. Correll and H. S. Gentry of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering for procuring the various plant specimens.

(9) Paper II, M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, submitted to *Anal. Chem.*

(10) The infrared assays were conducted by C. R. Eddy and M. E. Klumpp.

(11) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. This work was done as part of a coöperative arrangement between the Bureau of Plant Industry, Soils and Agricultural Engineering and the Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture, and the National Institutes of Health, Federal Security Administration.